159 Identification of *Burkholderia cepacia* complex species by SNuPE and LAMP analysis of histidine biosynthetic genes

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Respiratory infections are the main cause of morbidity and mortality in cystic fibrosis, and bacteria belonging to the *Burkholderia cepacia* complex (Bcc) are important infection agents. Bcc comprises nine species that are difficult to distinguish in routine clinical analysis. Nowadays a combination of molecular techniques is required for a correct identification of Bcc species; however, the application of these methods in clinical routine presents some difficulties. The aim of this study was to set up diagnostic methods based on SNuPE (Single Nucleotide Primer extension) and LAMP (Loop-mediated isothermal amplification). SNuPE allows detecting a single nucleotide polymorphism on a DNA target and to reduce misinterpretaion in Bcc species identification. Besides, it requires less time than the combination of techniques actually in use. LAMP is a very important tool in clinical fields since it requires simple equipments available in all laboratories.
Fifty-six strains both from environmental and clinical source and representative of nine BCC species were used.
We focused our attention on some genes of the histidine biosynthetic operon. Thus, a 3,500 bp DNA fragment from the 56 Bcc strains was PCR-amplified. The nucleotide sequence of each fragment was then determined and analyzed. In this way seven primers for the SNuPE technique were designed and used in multiplex reactions for obtaining the SNuPE profiles from Bcc strains.
Moreover, a set of primers for the LAMP was designed and used to amplify a his region of some Bcc reference strains.
Data obtained showed the high grade of applicability of both methods on Bcc members identification.
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160* Comparison of culture and molecular methods for detection and quantitation of aerobic and anaerobic bacteria in sputum from cystic fibrosis patients

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Introduction and Aims: We have previously shown by culture that the lungs of CF patients are colonised by both aerobic and anaerobic bacteria. The aim of this study was to compare culture and culture-independent techniques for determining the composition of bacterial communities in the sputum of CF patients.
Methods: Sputum was collected and processed using strict anaerobic techniques, prior to commencing and at the end of antibiotic therapy, from 26 adult CF patients admitted for treatment of an acute exacerbation. For culture detection, samples were plated on selective agars and bacteria quantified by viable count and identified by PCR and sequencing of 16S rRNA genes. For molecular detection, total DNA was extracted from sputum and bacterial species were identified by terminal restriction fragment length polymorphism analysis of PCR amplified 16S rRNA genes. Total bacterial abundance was determined by quantitative PCR.
Results: There was a good correlation between culture and molecular detection of the predominant aerobic pathogens (*P. aeruginosa* and *B. cepacia* complex). Anaerobic bacteria, from the genera *Prevotella* and *Veillonella*, were detected by both methods and were primarily associated with *P. aeruginosa* infection. Additional anaerobes were only identified by molecular detection. Bacterial community composition varied between patients but remained constant within most individuals before and after antibiotic treatment.
Conclusion: Molecular detection is both sensitive and accurate and may be a useful diagnostic technique for assessing infection in CF.

161 Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients

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There is strong evidence that culture-based methods detect only a small proportion of bacteria in the respiratory tracts of cystic fibrosis (CF) patients. Herein we have compared standard microbiological culture to molecular methods by the use of 16S rDNA amplification, cloning and sequencing in a series of CF sputa. Our purposes were to evaluate the bacterial diversity and to describe new pathogens colonizing CF lung. Twenty-five sputa from CF patients were cultured that yield 33 isolates (14 species) known to be pathogens during CF. For molecular cloning, 760 clones were sequenced, and 53 different bacterial species were identified including 16 species of anaerobes (30%). The mean number of species per sputum was 7.2±3.9. Identical results between culture and molecular data were observed in only 54.5% of cases. Discrepancies were numerous and demonstrate that accurate identification remains challenging. New or emerging bacteria not or rarely reported in CF patients were detected including Dolosigranulum pigrum, Dialister pneumosintes, and Inquilinus limosus. Our results demonstrate the complex microbial community in CF sputum, new or emerging bacteria may be detected in these patients, and anaerobes are probably an underestimated cause of CF lung pathology. Metagenomic analysis is urgently needed to better understand the complex communities in CF pulmonary infections.
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162 Comparison of the lung bacterial communities sampled from stable cystic fibrosis patients over time

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Over the past decade, the bacteria present in the lungs of cystic fibrosis (CF) patients have been shown to be highly diverse. Although the composition of these communities is increasingly well identified, very little is currently known about community dynamics. Such information would be of potentially profound clinical impact. The aim of this study was to determine the degree to which the bacterial community varies in the lungs of individual CF patients over time. In this study, the focus was on patients who were in clinically stable condition. A total of 15 sputa were collected from five CF patients over an average of 2 months. These were analysed by using culture-independent Terminal Restriction Fragment Length Polymorphism (T-RFLP) profiling of phylogenetically informative 16S rRNA genes extracted and amplified from these samples. Preliminary analysis has shown that the variation of the bacterial community in terms of the number and identity of bacterial species differed by less than 5% with the dominant bacterial species being conserved across samples taken from any one patient. The statistical significance of these findings in relation to the variation in abundance of the individual bacterial species and the implications in relation to treatment are also discussed.
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